Brown Marmorated Stink Bug Feeding and Potential for Biological Control in Minnesota Apple Orchards

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Dedication

To my mom, Kelly
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To my dad, Peter
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Abstract

There are many serious pests of apple crops in the United States (US), making production challenging for growers. A recent invasive insect in the US, the brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), has emerged as a serious pest of orchard crops with the highest economic losses to date occurring in the Mid-Atlantic region. Populations of *H. halys* are known to be increasing and spreading throughout the Midwest. If they continue to grow, the insect has potential to become a significant apple pest in the region. My research examined two areas to improve management of *H. halys* in the Midwest.

First, I compared three popular cold-hardy cultivars for risk of injury from *H. halys* in two-year field and laboratory experiments. Both field and laboratory experiments showed that the cultivars significantly varied in their risk for *H. halys* injury. One apple cultivar demonstrated a high risk for *H. halys* injury throughout all experiments and another seemed to be at risk at a later maturity date.

Secondly, I characterized the predator community of Minnesota apple orchards through season-long sampling over two years. The relative abundances and composition of the predators in orchards differed from previous studies in both proximate and distant states, which has direct implications for the level of biological control that can be achieved for *H. halys* in Minnesota. In addition, I compared the abundances of total predators and specific predator groups between apple cultivars sampled and found there to be significant differences across years.

Lastly, I examined the impact of natural enemies on *H. halys* in Minnesota apple orchards by conducting sentinel egg mass studies over two years. Overall predation was found to be low with no parasitism observed. This finding indicates that with current standard management practices in conventional Minnesota apple orchards, control of *H. halys* is likely to be low by existing natural enemies. The results from my thesis will provide information to fine tune IPM plans for *H. halys* to help create a more sustainable apple production system for growers in the Midwestern US.

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Introduction

Apple, *Malus domestica* Borkh. (Rosales:Rosaceae), is an important specialty crop in the United States (US) with an annual utilized production value of $3.6 billion as of 2017 (USDA-NASS 2018a). Apples are the most economically important fruit crop in Minnesota, with an annual harvest of 23 million pounds and an estimated market value of over $18 million (USDA-NASS 2018b). In addition, apple orchards in Minnesota contribute directly to tourism, offering pick-your-own opportunities and value-added products like cider (Norton 2019). The University of Minnesota has been a hub for cold-hardy apple breeding, producing many cultivars utilized throughout the Midwest (https://mnhardy.umn.edu/varieties/fruit/apples).

One of the many challenges faced in production of apple in the United States (US) is the attack by numerous insect pests, including native species such as the plum curculio, *Conotracheulus nenuphar* (Herbst) (Coleoptera: Curculionidae), and many invasive species such as the codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), the spotted tentiform leafminer, *Phyllonorycter blandcardella* (Fabricius) (Lepidoptera: Gracillariidae), and the apple maggot, *Rhagoletis pomonella* (Walsh) (Diptera: Tephritidae) (Krawczyk and Biddinger 2018; Bordelon et al. 2019; Wise 2019). Among the recent arrivals to the US, the brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), has the potential to be a devastating pest. *Halyomorpha halys* is an invasive insect native to east Asia that has spread to much of the United States, many European countries, southwest Russia, and

central Chile (Leskey and Nielsen 2018; Cianferoni et al. 2018). Since its first detection in Pennsylvania in 1996 (Hoebeke and Carter 2003), *H. halys* is now established in 44 US states and four Canadian provinces (www.stopbmsb.org/whereis-bmsb/state-by-state/). This insect is polyphagous feeding on over 100 different plant species, many of which are economically significant, and favor plant reproductive structures like fruits (Rice et al. 2014). In the US, *H. halys* has emerged as a serious pest of orchard crops with the highest economic losses to date occurring in Mid-Atlantic apple orchards (USAA 2010). In Minnesota, it was first discovered in 2010 in Ramsey County, with breeding populations since identified and detections radiating out into surrounding counties (Koch 2014; MDA 2019).

In the US, *H. halys* is known to complete one to two generations per year depending on the climate and temperature of the region (Nielsen et al. 2008; Govindan *unpublished*). *Halyomorpha halys* adults emerge from overwintering sites in the spring. Trees, shrubs, and ornamental plants near overwintering shelters often are the first plants on which they are found, but preference is given to taller plants and trees with sun exposure (Bergmann et al. 2013). As adult activity increases and mating, egg laying, and nymphal development occurs throughout the summer, *H. halys* can be found on a wide range of plant species (Bergmann et al. 2013). Furthermore, *H. halys* seems to exhibit 'host switching' behavior, like other stink bug species (Wang and Wang 1988; Fujisawa 2001; Tillman 2011; Lee et al. 2013, 2014; Wiman et al. 2015a), preferring certain

species of plants more than others, at particular times during the growing season (Funayama 2004; Bergmann et al. 2013; Rice et al. 2014).

In apples, feeding by *H. halys* and other stink bugs creates cork-like dead spots internally and discolored depressions externally and may render the fruit unmarketable (Brown 2003; Nielsen and Hamilton 2009; Acebes-Doria 2016; Shanovich 2019). Significant injury caused by *H. halys* in orchards can result in large economic losses. For example, in 2010, the apple industry alone estimated losses of about $37 million due to *H. halys* injury in the Mid-Atlantic region (USAA 2010). Based on the magnitude of damage inflicted by *H. halys* in the Mid-Atlantic, growers in recently-invaded areas such as Minnesota are gravely concerned (Wiman and Hoddle 2015).

Generalist insect herbivores, like *H. halys*, may exhibit strong and distinct intra-specific preferences among host plants related to nutritional quality, which could be due to secondary metabolites, morphological traits and other factors associated with the host plants (Bernays and Chapman 1994; Schoonhoven et al. 2005). However, differences are known to exist in the relative risk of cultivars for stink bug feeding and injury among apples grown in the eastern United States, which could be due to factors related to the cultivars' relative maturities at the time of exposure to *H. halys* (Brown et al. 2006; Brown and Short 2010). Several previous studies have found differences in the relative risk of cultivars to *H. halys* injury in blueberry, kiwi fruit, and soybean (Wiman et al. 2015b; Rich and Koch 2016; Lara et al. 2018; La Mantia et al. 2018), but information regarding the risk of *H. halys* injury to apple cultivars is lacking.

In orchard crops, the predominant method for managing *H. halys* has been application of foliar insecticides, with the majority of them being broad spectrum (Leskey et al. 2012a; Jentsch 2015; Bergh et al. 2016). Short et al. (2017) found that an economic threshold for *H. halys* is when cumulative captures of adults in any trap within the orchard or at the orchard border reaches ten *H. halys*. At the economic threshold, an effective insecticide should be applied as two alternate-row-middle sprays with seven days between applications to avoid populations from reaching the economic injury level (Short et al. 2017). These additional applications of broad-spectrum insecticides increase costs for growers, interfere with pre-existing integrated pest management (IPM) programs, and negatively affect natural enemy populations (Leskey et al. 2012a). The harmful effects of broad-spectrum pesticides on natural enemies have been well documented in many crop systems (Burn 1989; Theiling and Croft 1988, 1989; Epstein et al. 2000; Galvan et al. 2005; Deng et al. 2006; Mansfield et al. 2006; Rezaei et al. 2007; Sarvary et al. 2007; Johnson et al. 2008; Naranjo and Ellsworth 2009; Lu et al. 2012; Pezzini and Koch 2015).

Biological control is an component of IPM (Naranjo et al. 2015). Researchers have identified many different natural enemies attacking *H. halys* throughout the eastern US (Cornelius et al. 2016a, b; Ogburn et al. 2016; Morrison et al 2016; Pezzini et al. 2018). In apple orchards throughout the US, predator communities are known to be highly diverse, but the composition of communities varies between and within states (Oatman et al. 1964, McCaffrey and Horsburgh 1980, Carroll and Hoyt 1984, Miliczky and Horton 2005,

Biddinger et al. 2012, Horton et al. 2012, Morrison et al. 2016). Information on the predator community composition in Minnesota apple orchards is lacking, which utilizes different cultivars (Finnigan et al. 2000) and has a different climate from regions within most studies previously mentioned. In addition, natural enemy abundances are known to differ among crop cultivars (Berman and Tingey 1979) and information regarding this phenomenon is lacking in apple which could influence management decisions.

The focus of this thesis is to investigate feeding by *H. halys* and the potential for biological control in Minnesota apple orchards. In Chapter 1, I investigate the potential for *H. halys* to injure three popular cold-hardy apple cultivars grown in the Midwest. In Chapter 2, I characterize the composition of natural enemy communities, compare their abundances between cultivars, and assess their impact to *H. halys* sentinel eggs in Minnesota apple orchards. The results from my thesis will provide information to fine tune IPM programs for *H. halys* to help create a more sustainable apple production system for growers in the Midwest.

Chapter 1

Risk of cold-hardy apple cultivars to injury from brown marmorated stink bug (*Halyomorpha halys*)

Summary

An invasive species, the brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), has emerged as a serious pest of orchard crops in the United States with the highest economic losses to date in mid-Atlantic apple, *Malus domestica* Borkh. (Rosales: Rosaceae). If populations continue to grow and spread in the Midwest, *H. halys* has the potential to become a significant apple pest in the region. The purpose of this study was to assess the risk of injury from *H. halys* of several popular cold-hardy apple cultivars (Haralson, Honeycrisp and Zestar!®) grown in the Midwestern US utilizing both field no-choice tests and laboratory choice-tests. Results from the field no-choice tests revealed a greater risk for Honeycrisp from *H. halys* injury compared to Zestar!®. Results from the laboratory no-choice tests revealed a greater risk for injury by *H. halys* for Honeycrisp compared to Zestar!® at all maturities tested and a greater risk for Haralson compared to Honeycrisp at a late maturity date. These results serve as a preliminary step in assessing the potential impact of *H. halys* to different apple cultivars, which will help guide growers in cultivar selection and identifying what apple cultivars should be prioritized for scouting and management efforts.

Introduction

The brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), is an invasive insect native to east Asia that has spread to much of North America (44 U.S. states and four Canadian provinces), many European countries, southwest Russia, and central Chile (www.stopbmsb.org, Leskey and Nielsen 2018; Cianferoni et al. 2018). The insect is polyphagous feeding on over 100 different plant species, many of which are economically significant, and favors plant reproductive structures (Rice et al. 2014). Since its first detection in the U.S. in 1996 (Hoebeke and Carter 2003), *H. halys* has emerged as a serious pest of orchard crops with the highest economic losses to date occurring in orchards of Mid-Atlantic apple, *Malus domestica* Borkh (Rosales:Rosaceae) (USAA 2010).

In the U.S, the majority of apple production is located in the Pacific Northwest and eastern U.S., but the Midwest is also considered an important apple-growing region, accounting for approximately one-sixth of the production acreage in the country (USDA-NASS 2012). Apples are the most valuable fruit crop in the Midwest, with an annual harvest of over 444 thousand metric tons and an estimated market return of US$333 million (USDA-NASS 2017). *Halyomorpha halys*, which is currently considered a nuisance pest in most states of the Midwest, is commonly found in agricultural fields and orchards in Ohio and Michigan (Michel et al. 2015; Wilson et al. 2018), and is present in these agroecosystems in Wisconsin and Minnesota as well (UW-ENT 2016; Liesch 2018; MDA 2017). If populations continue to grow and spread, *H. halys* has great

potential to become a significant agricultural pest in the region. In the Midwest, cold-hardy apple cultivars are broadly utilized by growers due to the harsh winter climate (Finnigan et al. 2000). To date, the vast majority of research on *H. halys* feeding and injury to apples has been conducted on cultivars developed in and adapted to the eastern U.S. and Pacific Northwest.

Stink bugs are able to utilize different feeding strategies depending on the plant tissue targeted (Miles 1972; Hori 2000). Upon landing on a potential food source, Heteroptera typically enter an exploration phase that involves labial dabbing behavior characterized by repetitive touching of the food with the labial tip and external sensilla to assess acceptability (Backus 1985, 1988; Leopold et al. 2003). After the exploratory phase, if the food source is accepted, test probing follows that involves inserting the stylet into the plant tissue to assess the food quality for further digestion (Backus 1985). This phase can terminate abruptly or lead to exploratory feeding and finally feeding via excretion of watery saliva for extra-oral digestion (Backus 1985). Feeding is mediated by gel-like sheath saliva forming a hardened lining, called salivary sheaths, around the feeding stylet and plant tissues to prevent loss of juices from the site (Alhaddad et al. 2011; Medrano et al. 2011; Will et al. 2012). *Halyomorpha halys* exhibits sheath-feeding on fruits and seeds, which is common among the phloem and seed-feeding guilds of Hemiptera (Miles 1972). However, salivary sheaths can also form during the exploratory phase without ingestion taking place (Hollay et al. 1987; Wiman et al. 2014).

Injury resulting from stink bug feeding is due to mechanical damage by stylet probing (Depieri and Panizzi 2001), oxidative damage from digestive enzymes in saliva (Depieri and Panizzi 2001; Peiffer and Felton 2014), and/or the introduction of pathogens (Hollay et al. 1987; Medrano et al. 2007, 2009). Stink bug feeding in apple results in the formation of feeding punctures that are visible externally, necrotic tissue within fruits and nuts, and the subsequent development of depressions with or without discoloration on the surface (Brown 2003; Leskey et al. 2009). The watery saliva is believed to be responsible for causing the injury observed from *H. halys* feeding (Will et al. 2012). Feeding on apples early in the growing season, during cell division of the fruit, causes dimpling or "catfacing" on the surface of the fruit at the site of injury (Brown 2003).

The timing of exposure (i.e., early-mid season vs. mid-season to late-season) to *H. halys* has been found to affect the amount of injury to the apples; with fruit exposed during the mid- to latter portion of the season showing the most injury (Joseph et al. 2015). This phenomenon could simply be due to greater populations of *H. halys* adults and nymphs in orchards later in the season (Nielsen and Hamilton 2009a, b; Leskey et al. 2015; Bergh et al. 2016) and therefore more feeding occurring at this time. Additionally, a study comparing the injury at harvest of different life stages of *H. halys* on apple, found the highest percentage of injured fruit and number of injuries per fruit were caused by late-season adults as opposed to nymphs (Acebes-Doria et al. 2016).

However, cultivars of several crops are known to vary in their relative risk for injury by different stink bug species (Naresh and Smith 1984; Jones and

Sullivan 1978, 1979; Kester et al. 1984; Haddad and Louw 2006). Recent studies have reported similar trends for *H. halys* (Wiman et al. 2015b; Rich and Koch 2016; Lara et al. 2018; La Mantia et al. 2018). Differences are known to exist in the relative risk of cultivars to stink bug feeding and injury among apples grown in the eastern U.S. These differences could be due to factors related to the cultivars' relative maturities at the time of exposure; with fruit between 4-8 weeks before harvest incurring the most injury (Brown 2003; Brown et al. 2006; Brown and Short 2010). Variation in injury has also been observed with blueberry cultivars in a field no-choice setting, in which feeding and injury by *H. halys* differed among mid-season and late-season cultivars that were exposed to adults at different times throughout the growing season (Wiman et al. 2015b).

A recent study by Bergh et al. (2019), examined injury to apple cultivars that were exposed to *H. halys* adults for discrete intervals during each of the four weeks preceding their respective harvest dates. All but one cultivar examined exhibited a higher number of both external and internal injuries when exposed during the fourth week before harvest as compared to all other timings closer to harvest. However, the effect of cultivar on the amount of injury was not formally tested, as apple cultivars were not interspersed and replicated in an experimental design (Bergh et al. 2019).

We are unaware of studies that have looked at the relative risk for injury by *H. halys* of early-, mid- and late-season apple cultivars using both field no-choice and laboratory choice experiments. Furthermore, we are unaware of studies in apple that compare feeding by *H. halys* adults at different densities. In

this study, we examined the potential for *H. halys* to injure three popular cold-hardy apple cultivars grown in the Midwest. To accomplish this, we examined resulting internal and external injury at harvest from different densities of *H. halys* confined on apple cultivars in no-choice experiments under field conditions in August. We also examined salivary sheaths and resulting internal injury by *H. halys* from laboratory paired choice experiments with apple cultivars at different pre-harvest timings. Results of this research will build upon previous findings to help clarify the effect of *H. halys* feeding on resulting cultivar injury in cold-hardy apple during the late-season. These findings will help growers determine which cold-hardy apple cultivars should be prioritized for scouting and management efforts.

Materials and Methods

Apple cultivars

We utilized cold-hardy apple cultivars, Zestar!®, Honeycrisp and Haralson, developed by the University of Minnesota. These cultivars represent early season (i.e., ripening in late August – early September), mid-season (i.e., ripening in mid-late September), and late season (i.e., ripening in late September – late October) cultivars, respectively, for the region. Honeycrisp and Haralson share a common grandparent (Duchess of Oldenburg) and Haralson is one of the grandparents of Zestar!® (Cabe et al. 2005; Howard et al. 2017). All field and laboratory trials were conducted between early August and mid-September 2017 and 2018 to represent typical timing of feeding by adult *H. halys* and other stink

bugs in apple orchards (Brown et al. 2006; Nielsen and Hamilton 2009a, b; Leskey et al. 2015; Joseph et al. 2015; Acebes-Doria et al. 2016).

Insects

Halyomorpha halys were reared in a laboratory colony maintained at the University of Minnesota and originated from individuals collected in Wyoming, MN, U.S., in November 2016 and 2017. Adult *H. halys* were held in mixed-sex groups of 50-60 individuals in mesh cages (34 × 34 × 61 cm) (BioQuip Products, Inc., Rancho Dominques, CA) at ~25 ºC, ~70% RH, and 16:8 (L:D) photo regime (Niva and Takeda 2003). A flat (23 × 23 cm) of organic vegetative-stage green bean (*Phaseolus vulgaris* L.) (Fabales: Fabaceae) plants grown in potting soil were placed in the bottom of each cage for use as an oviposition substrate (Dieckhoff et al. 2017). *Halyomorpha halys* were fed organic carrots (*Daucus carota* subsp. sativus (Hoffm.) Schübl. & G. Martens) (Apeales: Apiaceae), green bean pods, and sunflower (*Helianthus annuus* L.) (Asterales: Asteraceae) and soybean (*Glycine max* (L.) Merr.) (Fabales: Fabaceae) seeds placed on top of the cages (Funayama 2006; Dingha and Jackai 2017). Water was provided daily by misting each cage with deionized water. Beginning in mid-May of each year, egg masses were collected daily from the underside of the green bean leaves. Egg masses were placed individually in Petri dishes (55 mm) lined with a half piece of filter paper, held in a growth chamber (Percival Scientific, Inc., Perry, IA) under the same environmental conditions as the laboratory colony, and provided with a few drops of deionized water daily. Upon molting to the second instar, nymphs were transferred to mesh cages (61 × 61 × 91 cm) and provisioned with the same

diet and substrate as described for the adults. Upon eclosing to adults, individuals were sexed and put into separate cages and starved for 24 h prior to the initiation of experiments.

Measurements of apple maturity

In 2017, for both the field and laboratory experiments, we recorded measurements of the percentage of soluble solids (i.e., Brix) using a Sugar/Brix Refractometer 0 to 32% with Automatic Temperature Compensation (Sper Scientific, Scottsdale, AZ) and starch content using the starch-iodine test (Blanpied and Silsby 1992) for undamaged apples (n=7 or 8) of each cultivar from the same trees as the apples used in the experiments. In 2018, for both field and laboratory experiments, we recorded measurements of soluble solids, starch-iodine indexes (as described above) and fruit firmness, measured as overall average hardness (OAH) in kilograms force (kgf) with a Mohr Digi-Test-2 Series (MDT) Computerized Penetrometer and Texture Analyzer (MOHR, Richland, WA).

Field no-choice tests

Methods for field no-choice tests were adapted from Wiman et al. (2015b) and Acebes-Doria et al. (2016). Field no-choice feeding tests were conducted in early August 2017 and 2018 at the University of Minnesota Research and Outreach Center near Rosemount, Minnesota, USA. The study site consisted of a 0.1-hectare fenced plot containing 17 apple trees (six trees each of Zestar!® and Haralson and five of Honeycrisp) planted in 2012 in a randomized block design with trees spaced 6.1 meters apart. Disease and pest management in the orchards

followed standard recommendations for this region (McCamant 2007). Insecticide and fungicide applications ceased three weeks prior to initiation of field experiments in 2017 and 2018.

In early July of each year, trees were surveyed for pairs of apples that were less than 15 cm apart on branches, which were then enclosed in mesh sleeve cages (two apples per cage) to prevent injury from other insects prior to the tests. Cages used for the tests were cylindrical sleeve cages (45 cm long, and 25 cm in diameter), constructed from no-see-um mesh (Quest Outfitters, Sarasota, FL) with one open end that could be closed around a branch with a drawstring.

On 11 August 2017, cages on each tree were randomly assigned one of two treatments: infestation with five adult *H. halys* or uninfested (i.e., control). In total there were six infested cages and six uninfested cages per cultivar, with the exception of Zestar!® which had seven infested cages. *Halyomorpha halys* used for this experiment were all adult females that eclosed between 5 and 6 August 2017 (rearing described above).

In 2018, due to poor fruit set for the entire orchard, methods were modified from those of the previous year and the cultivar Haralson had to be excluded from the experiment. Infestation time for 2018 was chosen based on soluble solids and starch-iodine indexes (methods described above) in order to match the same maturity stage of the apples in the 2017 field no-choice test. On 8 August 2018, cages on each tree were randomly assigned one of three treatments: infestation with five adults *H. halys*, infestation with two adult *H. halys*, or uninfested (i.e., control). For each cultivar, there was a total of seven cages with

each *H. halys* density. *H. halys* used for this experiment were all adult females that eclosed between 3 and 6 August 2018 (rearing described above).

In both years, cages were checked at 48 h for dead *H. halys*, which were replaced with live individuals as needed to maintain treatment levels. After 96 h, all *H. halys* were removed from the cages and the cages were left on the apples until the respective harvest date for each cultivar. Timing of harvest was determined by estimated cultivar-specific harvest dates in the region (Schwallier and Irish-Brown 2017, 2018; MAGA 2019) and by measures of soluble solids and starch-iodine indexes (Blanpied and Silsby 1992). In 2017, Zestar!® was harvested on 30 August, Honeycrisp on 15 September, and Haralson on 10 October. In 2018, Zestar!® was harvested on 28 August and Honeycrisp on 12 September.

Methods for evaluating injury to apples were adapted from Joseph et al. (2015) and Acebes-Doria et al. (2016). After harvest of each cultivar, apples were stored in the laboratory at 4 ºC and evaluated within 24 h. External injury was recorded as the number of depressions, with or without discoloration, on the surface of the apples. Internal injury was recorded as the number of areas of necrotic tissue after peeling the skin and cutting each fruit into ~25-mm thick slices. Counts of external depressions and internal necrotic spots were recorded for each apple. Measures of injury were averaged across the two apples in each cage. Calcium-related deficiency disorders in apple, especially bitter pit (i.e., cork spot), can be confused with stink bug feeding as they cause similar symptoms on fruit, including exterior depressions and discolorations and internal cork spots (Brown 2003, Leskey et al. 2009). Honeycrisp is known to be especially

susceptible to bitter pit from calcium deficiency (Telias et al. 2006), which causes sunken brown discolored spots externally and diffuse brown cork spots internally (Brown 2003). However, late-season stink bug damage is distinguishable from bitter pit due to the presence of a stylet puncture and/or salivary sheath on the surface of the skin in stink bug damage (Brown 2003; Joseph et al. 2015). Additionally, the stink bug damage can have a circular appearance exteriorly and internally the necrotic tissue is uniform and contiguous with the apple skin (Brown 2003).

Laboratory choice-tests

Choice-tests were performed in the laboratory at ~25 ºC, ~70% RH, and 16:8 (L:D) photo regime. The experimental setup included 24 clear storage boxes (i.e., arenas) (35 × 21 × 12 cm) (Sterilite Corporation, Townsend, MA) positioned with the lids facing downward. Each arena contained two apples, one on either side of the arena. Each pairwise combination of cultivars, Zestar!® and Honeycrisp; Zestar!® and Haralson; and Honeycrisp and Haralson, was replicated eight times. Each arena received four adult *H. halys,* either all male or all female, that were randomly assigned to each replication.

In 2017, *H. halys* adults were placed in the center of containers on 21 August and allowed to feed for 72 h. All *H. halys* adults used in this study eclosed on 8 August 2017. Apples used in these experiments were picked on 21 August from the study site at Rosemount, MN as described in the field no-choice tests.

In 2018, three separate sets of choice-test tests were performed from early August through early September with eight replications of pairwise combinations

of cultivars per experiment. The first set of choice-test tests was initiated on 8 August with fruit from all cultivars being unripe. Relative maturity was assessed by monitoring soluble solids and starch-iodine indexes beginning in July. Adult *H. halys* used in these choice tests eclosed on 6 August. The second set of choice-tests were initiated on 22 August with Zestar!® being ripe, and Honeycrisp and Haralson being unripe; this test reflected the same timing used for the 2017 choice experiment. Adults used in these choice tests eclosed between 15-20 August. The third set of choice-tests was initiated on 11 September with Honeycrisp being ripe and Haralson being unripe. Adults used in these choice tests eclosed on 4 September. In this set of choice tests, the cultivar Zestar!® was not used because it was overripe and harvesting would have already been completed for the season. Due to the poor fruit set in the orchard at Rosemount, MN, apples for these tests were obtained from a commercial apple orchard in White Bear Lake, MN. That orchard followed conventional management practices for the area and apples for the experiment were picked on the same day as the initiation of each corresponding experiment. All apples were thoroughly rinsed with tap water before use in the choice-tests (Kamminga et al. 2009). For each of these sets of choice-tests, containers were checked periodically to replace any dead individuals.

Following the 72 h for each choice-test, insects were removed. Fruits were then inspected for salivary sheaths and internal injury. The number of salivary sheaths on each apple was recorded. Salivary sheaths remain in the plant tissue after stink bug feeding and can serve as an indicator of probing or feeding activity

by stink bugs (Bowling 1979; Bowling 1980; Lye and Story 1988; Simmons and Yeargan 1988; Panizzi et al. 1995; Brennan et al. 2009; Cira et al. 2017) and have been used to effectively predict crop injury by stink bugs (Bowling1979, 1980; Viator et al. 1983; Barbour et al. 1990; Bundy et al. 2000). Furthermore, salivary sheaths have been used to infer the relative risk of *H. halys* feeding and injury among cultivars in several studies (Wiman et al. 2015b; Lara et al. 2018). However, we recognize that food consumption (i.e., ingestion) and risk cannot necessarily be inferred from salivary sheath counts alone as species and stage-specific differences in the relation between consumption and salivary sheaths have been recorded for stink bugs feeding on cotton (Zeilinger et al. 2015). We are using salivary sheaths to measure the number of stylet penetrations or probes, not the amount of plant biomass consumed.

The apples were then held in a refrigerator (6 ºC) for injury to develop (Brown and Short 2010). After two weeks in the refrigerator, the apples were evaluated internally for necrosis as in the field no-choice tests. External depressions were excluded as a measure of injury in laboratory choice-tests as very few developed on the fruits under laboratory conditions, which may be due to lack of UV radiation or other environmental factors (Brown and Short 2010).

Data Analyses

All analyses were conducted with R version 3.4.4 (R Core Team 2018a). Measurements of apple maturity (i.e., Brix, starch-iodine index and firmness) were analyzed separately for each field choice test and no-choice test as linear models using one-way analysis of variance (ANOVA) with cultivar as the

predictor variable (package, code: stats, *aov*; R core team 2018b). Means were compared by obtaining least-square means (package, code: lsmeans, *lsmeans*; Lenth 2016) adjusted for Tukey's HSD test. Assumptions of normally distributed residuals and constant variance were checked for each linear model using qqplots, residuals plots. No transformations of the response variable were needed.

For the field no-choice test in 2017, injury types (i.e., depressions and necrotic spots) were analyzed separately as linear models using one-way ANOVAs with cultivar as the predictor variable (package, code: stats, *lm*; R core team 2018b). Means were compared by obtaining least-square means (package, code: lsmeans, *lsmeans*; Lenth 2016) adjusted for Tukey's HSD test. In 2018, injury types (i.e., depressions and necrotic spots) were analyzed separately using two-way analysis of variance ANOVAs with cultivar, *H. halys* density, and their interaction as the predictor variables (package, code: stats, *lm*; R core team 2018b). Non-significant ($P > 0.05$) cultivar*density interaction terms were removed from linear models. Assumptions of normally distributed residuals and constant variance were checked for each linear model using qqplots, residuals plots. No transformations of the response variable were needed.

For the laboratory choice-tests, injury indicators (i.e., salivary sheaths and necrotic spots) were analyzed separately, comparing differences between cultivars of each pairwise combination using one-sample t-tests (package, code: stats, *t.test*; R core team 2018b); in which the difference between each individual pair of cultivars was compared to zero (i.e., indicative of no difference). Assumptions of normally distributed residuals and constant variance were checked for each linear

model using qqplots and residual plots. Counts of salivary sheaths were square root transformed to normalize variance in comparisons between Zestar!® and Haralson and between Honeycrisp and Haralson from 11 August 2018, the comparison between Zestar!® and Haralson from 22 August 2018, and all comparisons from 11 September 2018. Counts of necrotic spots from 11 September 2018 were also square root transformed.

Results

Measurements of apple maturity

For both years in each experiment, apple cultivars differed in Brix levels and starch-iodine indexes, with Zestar!® consistently being the highest followed by Honeycrisp and Haralson (Table 1.1). No difference in firmness was observed on 8 August 2018 between any of the cultivars (Table 1.1). On 21 August 2018, Zestar!® was found to be less firm than Honeycrisp and Haralson (Table 1.1). On 11 Sept 2018, Honeycrisp was less firm than Haralson (Table 1.1).

Field no-choice tests

In 2017, after exposure of apples to five *H. halys* adults, the average number of depressions differed between cultivars (F=4.49; df=2, 17; *P*=0.0272). There was an average of 7.9±1.8 (±SEM) depressions per fruit on Honeycrisp, which was more than twice that of Zestar!® at 2.9±0.5 depressions per fruit (Figure 1.1a). The average number of necrotic spots per fruit also differed between cultivars (F=3.92; df=2, 17; *P*=0.0406). Honeycrisp again had more than twice the amount as Zestar!®, with an average of 10.4±2.3 compared to 4.1±1.0

necrotic spots per fruit, respectively (Figure 1.1b). Injury observed on Haralson did not differ from the other two cultivars (Figure 1.1a, b).

In 2018, when exposed to densities of two or five adult *H. halys*, the average number of depressions per fruit was greater for Honeycrisp compared to Zestar!® (F=8.95; df=1, 25; *P*=0.0061). At a density of two adult *H. halys*, Honeycrisp incurred 5.4±1.3 depressions per fruit, which was more than twice that of Zestar!® at 2.1±0.5 depressions per fruit (Figure 1.1c). At a density of five adult *H. halys*, Honeycrisp incurred 10±2.6 depressions per fruit, which was more than three times the amount Zestar!® incurred at 2.9±0.8 depressions per fruit (Figure 1.1c). However, the number of depressions per fruit did not differ between densities of *H. halys* (F=0.95; df= 1, 25; *P*=0.3381) (Figure 1.1c), meaning there was not a significant increase in the amount of depressions in going from two to five adult *H. halys*. The mean number of necrotic spots per fruit was greater for Honeycrisp than Zestar!® (F=13.21; df= 1, 25; *P*=0.0013). At a density of two adult *H. halys*, Honeycrisp incurred 12.6±3.2 necrotic spots per fruit which was more than three times that of Zestar!® at 3.7±0.8 necrotic spots per fruit (Figure 1.1d). At a density of five adult *H. halys*, Honeycrisp incurred 21±5.8 necrotic spots per fruit which was more than five times that of Zestar!® at 4.7±1.6 (Figure 1.1d). Again, there was no difference between densities of *H. halys* for the number of necrotic spots per fruit (F=1.86; df= 1, 25; *P*=0.1850) (Figure 1.1d).

Laboratory choice-tests

In 2017, there was a difference in the average number of salivary sheaths per fruit between Honeycrisp and Zestar!® (t= 2.99; df= 7; *P*= 0.0203); with Honeycrisp exhibiting approximately 15 times as many salivary sheaths per fruit as Zestar!® at 14.8 ±5.02 and 1.1±0.5, respectively (Figure 1.2a). Similarly, the average number of necrotic spots per fruit was greater on Honeycrisp than Zestar!® (t= 6.23; df= 7; *P*= 0.0004); with Honeycrisp exhibiting approximately 25 times more than Zestar!® at 6.2±1.0 and 0.2±0.2, respectively (Figure 1.3a). Haralson had more salivary sheaths per fruit than Zestar!® (t= 6.70; df= 7; *P*= 0.0003), with Haralson exhibiting approximately 115 times as many salivary sheaths per fruit as Zestar!® at 34.7±9.4 and 0.2±0.2, respectively (Figure 1.2a). Haralson also had more necrotic spots per fruit than Zestar!® (t= 9.69; df= 7; *P*= <0.0001), with Haralson exhibiting approximately 33 times more than Zestar!® at 8.3±2.2 and 0.1±0.1, respectively (Figure 1.3a). There was no difference between the number of salivary sheaths per fruit on Honeycrisp and Haralson (t= -1.14; df= 7; *P*= 0.2906) (Figure 1.2a). Furthermore, there was no difference between the average number of necrotic spots per fruit on Honeycrisp and Haralson (t= -0.05; df= 7; *P*= 0.6224) (Figure 1.3a).

In 2018, for the choice-tests initiated on 8 August, there was no difference between the average number of salivary sheaths per fruit between Honeycrisp than Zestar!® (t= 1.61; df= 7; *P*= 0.1523) (Figure 1.2b). There were more necrotic spots per fruit on Honeycrisp than Zestar!® (t= 2.44; df= 7; *P*= 0.0447), with Honeycrisp exhibiting almost twice as many necrotic spots per fruit as Zestar!® at 37.0±8.0 and 19.1±6.0, respectively (Figure 1.3b). There again was a difference in

the average number of salivary sheaths per fruit between Haralson and Zestar!® (t= 2.59; df= 7; *P*= 0.0359), with Haralson exhibiting twice as many necrotic spots per fruit as Zestar!® at 37.1±10.4 and 16.9±5.6, respectively (Figure 1.2b). However, there was no difference in the mean number of necrotic spots per fruit between Haralson and Zestar!® (t= 1.32; df= 7; *P*= 0.2269) (Figure 1.3b). There were no differences observed between the mean number of salivary sheaths per fruit (t=1.13; df=7; *P*=0.2940) (Figure1. 2b) nor necrotic spots per fruit between Honeycrisp and Haralson (t= 0.29; df= 7; *P*= 0.7787) (Figure 1.3b).

For the choice-tests initiated on 21 August 2018, there was a difference in the average number of salivary sheaths per fruit between Honeycrisp than Zestar!® (t=2.71; df= 7; *P*= 0.0304); with Honeycrisp exhibiting more than twice salivary sheaths per fruit as many as Zestar!® at 19.4±4.3 and 8.8±2.8, respectively (Figure 1.2c). There was also a difference between the average number of necrotic spots per fruit for Honeycrisp and Zestar!® (t= 3.74; df= 7; *P*= 0.0072); with Honeycrisp exhibiting more than twice as many necrotic spots per fruit as Zestar!® at 18.8±1.9 and 8.0±2.8, respectively (Figure 1.3c). There were no differences in the average number of salivary sheaths per fruit between Haralson and Zestar!® (t= 1.08; df= 7; *P*= 0.3173) (Figure 1.2c), nor in the average number of necrotic spots per fruit between these cultivars (t= 1.67; df= 7; *P*= 0.1387) (Figure 1.3c). There were again no differences in the average number of salivary sheaths per fruit (t= -0.75; df= 7; *P*= 0.4771) (Figure 1.2c), nor necrotic spots per fruit (t= 1.36; df= 7; P= 0.2152) (Figure 1.3c) between Honeycrisp and Haralson.

Finally, for the choice-tests initiated on 11 September 2018, there was a difference in the average number of salivary sheaths per fruit between Honeycrisp and Haralson (t= -2.60; df= 7; *P*= 0.0353); with Haralson exhibiting approximately 6 times as many salivary sheaths per fruit as Honeycrisp at 29.1±8.6 and 4.8±1.5, respectively (Figure 1.2d). There was also a difference in the average number of necrotic spots per fruit between Honeycrisp and Haralson (t= -3.21; df= 7; *P*= 0.0148); with Haralson exhibiting approximately 4 times as many as Honeycrisp at 13.3±3.9 and 3.1±0.6, respectively (Figure 1.3d).

Discussion

Apple cultivars are known to differ in their risk for injury from stink bugs and the relative maturity of an apple cultivar appears to be the determining factor for stink bug injury in the field; with fruit between 4-8 weeks before harvest incurring more injury (Brown 2003; Brown et al. 2006; Brown and Short 2010; Bergh et al. 2019). However, information regarding the risk of apple cultivars for *H. halys* injury is generally lacking. To our knowledge, this is the first study to directly compare cold-hardy apple cultivars for risk of injury from *H. halys*. Our results provide strong evidence that the risk of injury to apples from *H. halys* differs by cultivar and that this risk may be related to the relative maturity of the fruit at the time of exposure to *H. halys*.

Across our field no-choice tests and laboratory choice tests, Honeycrisp consistently incurred more injury than Zestar! ®. Across all of these studies, Honeycrisp ranged from 3.71-5.43 weeks from harvest and had lower Brix and starch-iodine indexes than Zestar! ®, while Zestar! ® ranged from 1-2.86 weeks

from harvest (Table 1.1). These findings are consistent with those of previous studies showing that apples 4-8 weeks from harvest appear to be at higher risk for stink bug injury than those that are less than 3-4 weeks from harvest (Brown 2003; Brown et al. 2006; Brown and Short 2010; Bergh et al. 2019). Therefore, we conclude that Honeycrisp apples are at greater risk than Zestar!® apples throughout August.

Interestingly, Haralson, which was the latest maturing among the three cultivars, did not consistently incur greater injury than the other cultivars. In our field no-choice tests, measure of injury per fruit for Haralson did not differ from either Honeycrisp or Zestar!®, even though Haralson ranged from 8.57-8.86 weeks away from its harvest date and had lower Brix levels and starch-iodine indexes than both other cultivars (Table 1.1) . In laboratory choice tests, Haralson exhibited more salivary sheaths per fruit than Zestar!® in two trials and more necrotic spots in only one trial. However, Haralson did exhibit more salivary sheaths and necrotic spots per fruit than Honeycrisp in the September timing, when Honeycrisp was less than a week from its harvest date and Haralson was 4 weeks away (Table 1.1). Across our laboratory choice tests, Haralson had lower starch-iodine indexes and Brix levels than Honeycrisp. However, the firmness of both cultivars was not different until the choice-test performed in September, when Honeycrisp became less firm than Haralson. Therefore, we suspect that Haralson's relative risk for *H. halys* injury increases as Honeycrisp reaches ripeness, at which time Honeycrisp becomes relatively less at risk. This finding confirms that the risk of a cultivar for *H. halys* injury can change over time based

on the relative maturity of the cultivar and may also depend on what other cultivars are present within an orchard. Similarly, it has been suggested that the preferences of another pest of apple, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), depend on apple firmness throughout the season (Stoeckli et al. 2011).

Likewise, previous studies have also found exceptions to this window of 4-8 weeks before harvest for fruit to incur more injury from stink bug feeding. Brown et al. (2006) found a few cultivars with lower levels of injury among mid- and late-season cultivars, which may suggest genetic variation in the risk of incurring stink bug injury among mid- and late-season cultivars. A similar phenomenon was observed by Wiman et al. (2015b) with a mid-season blueberry cultivar, Elliott, that experienced consistently low levels of salivary sheaths and necrosis in field no-choice tests with *H. halys* compared to other mid- and late-season cultivars at different timings and densities throughout the growing season. Considering that Haralson is one of the grandparents of Zestar!® (Cabe et al. 2005), there potentially may not be a large enough genetic difference between Zestar!® and Haralson that contributes to Haralson not consistently exhibiting a greater risk for *H. halys* injury than Zestar!® at different developmental stages.

The bug densities tested in the trials also returned interesting results. Unexpectedly, in 2018, there was not a difference in the number of depressions or necrotic spots at a density of five *H. halys* adults per cage compared to two *H. halys* adults per cage. This finding implies that a relatively low level of *H. halys* adults may be sufficient to cause significant injury to fruit. Similarly, other studies

have not always found differences in crop injury between density treatments of *H. halys* in field no-choice tests (Wiman et al. 2015b; Koch and Rich 2015). For example, Koch and Rich (2015) did not observe differences in the percent of injured soybean seeds between density treatments of two and four *H. halys* nymphs per 0.33 m of row but did at a density of eight *H. halys* nymphs per 0.33 m of row. Likewise, Wiman et al. (2015b) did not observe differences in the percent of necrotic or discolored blueberries for the majority of cultivars until density treatments reached a high of 10 *H. halys* adults per cluster. This lack of difference between density treatments could be due to intraspecific competition (Hughes and McKinlay 1987). Therefore, future studies should utilize a wider range of density treatments to better characterize apple response to *H. halys* density.

Interestingly, in field no-choice tests across years, apples tended to show numerically more internal (i.e., necrotic spots) than external injuries (i.e., depressions). This phenomenon was also recently reported by Bergh et al. (2019). This lack of a 1:1 ratio of depressions to necrotic spots could complicate scouting and management decisions for the pest. In contrast, in our laboratory choice tests, we generally observed greater numbers of salivary sheaths than internal injuries per fruit. This observation was especially apparent with Haralson apples, having close to twice as many salivary sheaths as necrotic spots in some trials and comparisons (Figure 1.2a, b, c). For example, in the earliest maturity choice-test (8 Aug 2018), Haralson apples incurred a higher number of salivary sheaths than Zestar!® apples (Figure 1.2b), but the number of necrotic spots did not differ

between the two cultivars (Figure 1.3b), which could be an indication that Haralson may be of lower food quality for *H. halys* but is still attractive when compared to Zestar!®. As described previously, 'test probing' is an exploratory behavior of stink bugs that can result in the formation of salivary sheaths without actual feeding occurring (Hollay et al. 1987, Wiman et al. 2014). Wiman et al. (2015b) also observed this phenomenon with a mid-season blueberry cultivar, Bluecrop, experiencing high levels of salivary sheaths, but comparably low levels of necrosis.

The findings of our study could be used to inform future planting decisions and fine tune integrated pest management plans for *H. halys* in cold-hardy apple production. Planting cultivars, such as Zestar!®, that mature earlier or exhibit less injury overall could help minimize injury from *H. halys*. Scouting efforts and chemical treatments could also potentially be focused more on mid to late-season maturing cultivars or more susceptible cultivars like Honeycrisp. As *H. halys* populations increase in the Midwest, more studies and direct field observations will be needed to understand how insect phenology, feeding behavior and development of cold-hardy apple cultivars will impact fruit injury within orchards containing many different cultivars.

Table 1.1. Maturity characteristics (mean ± SEM) and weeks to harvest of apple cultivars used in laboratory and field tests.

Test	Date[b]	Metric	Cultivar [a]			F	df	*P*
			Zestar!®	Honeycrisp	Haralson			
Field	11 Aug 2017	Brix (%)	11.00±0.19**a**	9.13±0.18**b**	8.13±0.35**c**	75.34	2, 20	<0.001
		Starch-iodine index	2.5±0.11**a**	2±0.11**b**	1.50±0.10**c**	321.80	2, 20	<0.001
		Weeks to harvest[c]	2.71	5.43	8.57	---	---	---
Lab	21 Aug 2017	Brix (%)	13.19±0.43**a**	10.56±0.14**b**	8.63±0.25**c**	58.48	2, 20	<0.001
		Starch-iodine index	3±0.09**a**	2.5±0.10**b**	2±0.09**c**	489.54	2, 20	<0.001
		Weeks to harvest	1.29	3.43	7.00	---	---	---
Field	8 Aug 2018	Brix (%)	12.19±0.16**a**	9.69±0.25**b**	---	70.89	1, 13	<0.001
		Firmness (kgf)	4.43±0.14**a**	6.13±0.10**b**	---	103.76	1, 13	<0.001
		Starch-iodine index	2.5±0.10**a**	2±0.11**b**	---	40.39	1, 13	<0.001
		Weeks to harvest	2.86	5.00	---	---	---	---
Lab	8 Aug 2018	Brix (%)	11.44±0.20**a**	9.43±0.34**b**	7.53±0.21**c**	64.69	2, 20	<0.001
		Firmness (kgf)	5.70±0.19**a**	6.21±0.09**a**	5.70±0.18**a**	3.02	2, 20	0.071
		Starch-iodine index	2.5±0.09**a**	2±0,10**b**	1.5±0.09**c**	344.93	2, 20	<0.001
		Weeks to harvest	2.86	5.00	8.86	---	---	---
Lab	21 Aug 2018	Brix (%)	12.07±0.13**a**	9.88±0.16**b**	8.00±0.27**c**	76.43	2, 20	<0.001
		Firmness (kgf)	4.71±0.20**a**	5.80±0.12**b**	5.36±0.09**b**	14.58	2, 20	<0.001
		Starch-iodine index	3±0.10**a**	2.5±0.08**b**	2±0.09**c**	346.47	2, 20	<0.001
		Weeks to harvest	1.00	3.71	6.57	---	---	---
Lab	11 Sept 2018	Brix (%)	---	12.88±0.16**a**	9.25±0.25**b**	150.95	1, 13	<0.001
		Firmness (kgf)	---	4.69±0.08**a**	5.03±0.10**b**	7.12	1, 13	0.018
		Starch-iodine index	---	3.5±0.08**a**	2.5±0.09**b**	225.00	1, 13	<0.001
		Weeks to harvest	---	<1.00	4.00	---	---	---

[a] Letters within each row separate the adjusted variances/intercepts based on Tukey's HSD post hoc test ($P < 0.05$)

[b] Date *Halyomorpha halys* placed in cages/arenas for field no-choice tests and arena for laboratory choice tests

[c] Weeks to harvest for field no-choice tests were determined by subtracting the date *Halyomorpha halys* were placed in cages from the date the apples were harvested and for laboratory choice tests, were determined by subtracting the date *Halyomorpha halys* placed in arenas from the date the apples were harvested in field no-choice tests; for Haralson, the harvest date used was obtained from the commercial orchard that the apples originated from.

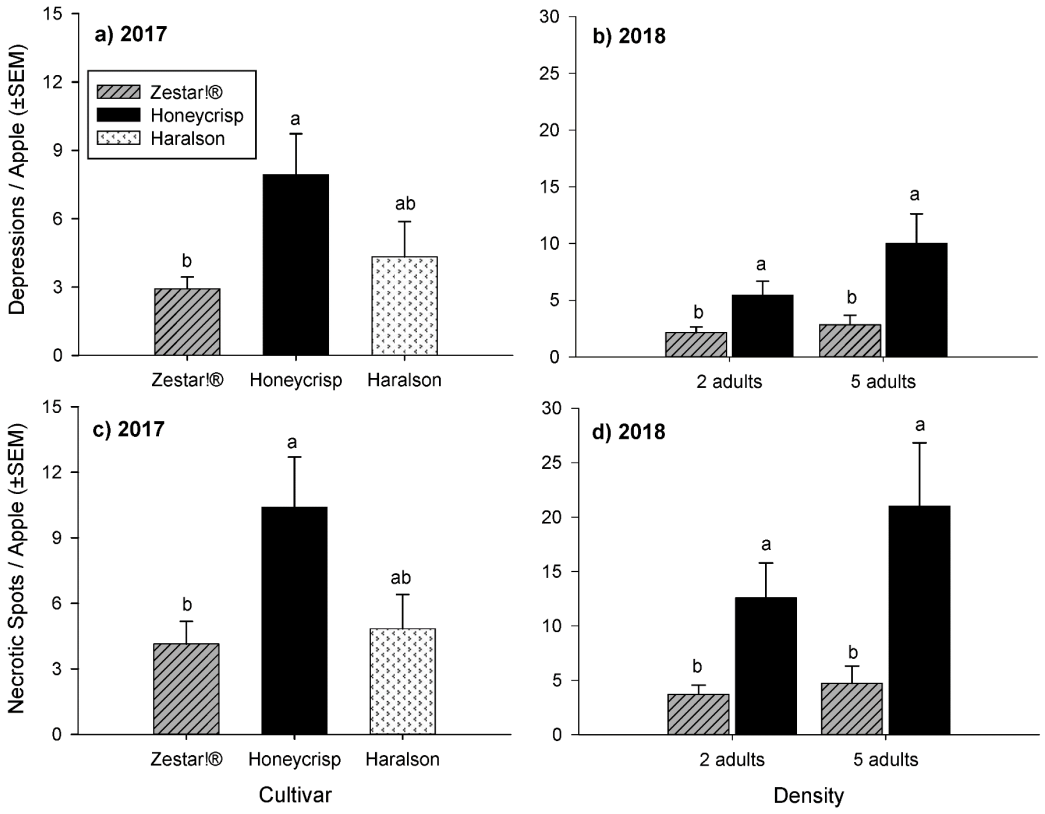

Figure 1.1. Mean number of depressions (a and b) and necrotic spots (c and d) per apple at respective time of harvest for each cultivar after exposure to *H. halys* adults for 96 h in field no-choice tests in 2017 and 2018. Density treatments in 2018 indicate 2 adult *H. halys* per cage and 5 adult *H. halys* per cage, with two apples per cage. Different letters indicate a significant difference between cultivars and densities at $P < 0.05$.

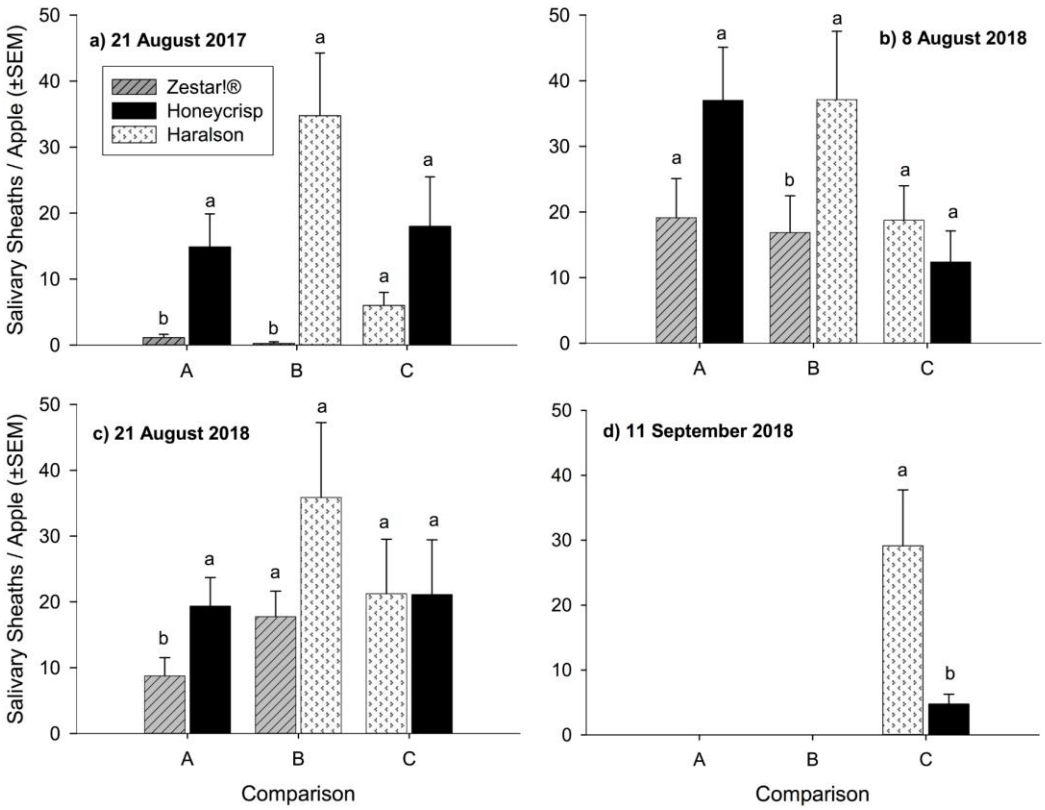

Figure 1.2. Number of salivary sheaths per apple over 4 harvest dates in 2017 and 2018 (a-d). Data represent paired-choice tests of three cultivar comparisons after exposure to *H. halys* adults for 72 h and a following two weeks for development of damage. Comparison A represents the apple cultivars Zestar!® and Honeycrisp, comparison B represents Zestar!® and Haralson and comparison C represents Honeycrisp and Haralson. Different letters indicate a significant difference between cultivars and densities at $P < 0.05$.

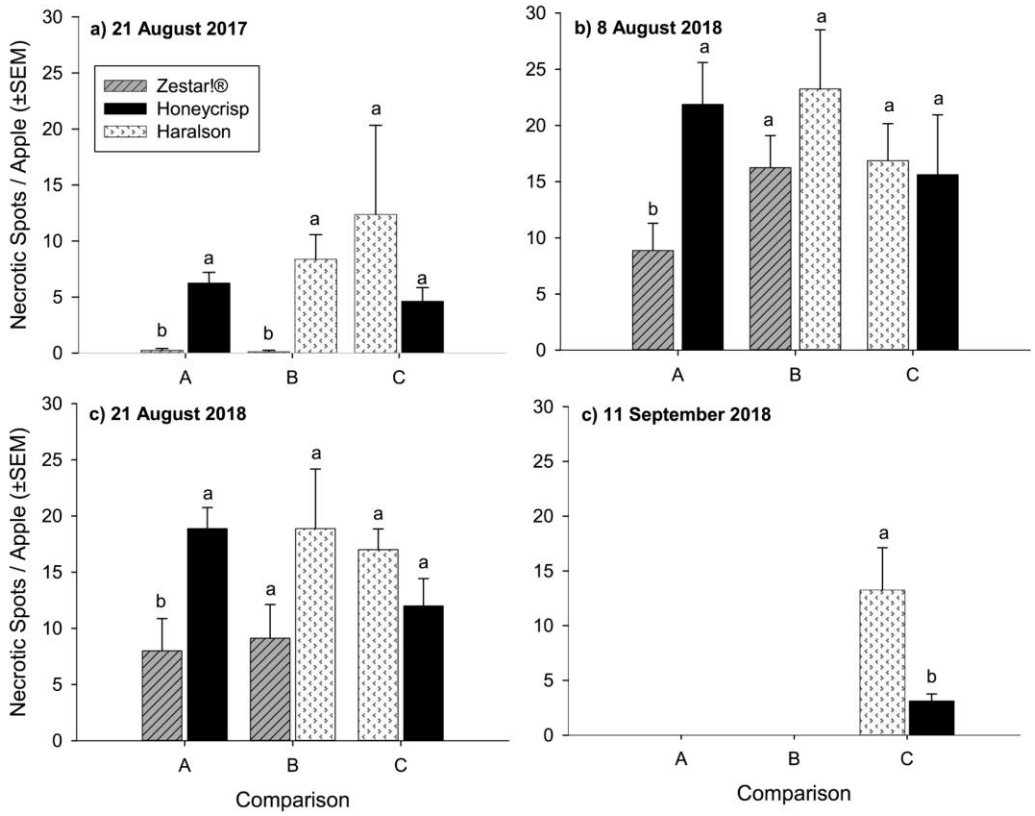

Figure 1.3. Mean number of necrotic spots per apple over four maturity dates in 2017 and 2018 (a-d). Data represent paired-choice test of three comparisons after exposure to *H. halys* adults for 72 h and a following two weeks for damage development. Comparison A represents the apple cultivars Zestar!® and Honeycrisp, comparison B represents Zestar!® and Haralson and comparison C represents Honeycrisp and Haralson. Different letter indicates a significant difference between cultivars and densities at $P < 0.05$.

Chapter 2

Natural enemy community composition, abundance and their impact on *Halyomorpha halys* eggs in Minnesota apple orchards

Summary

Halyomorpha halys is a polyphagous invasive insect to the United States (US), which feeds on many major crops, including apple. State-wide monitoring in Minnesota has shown continued increase of *H. halys* populations and occurrence of this pest in apple orchards. Potential arthropod natural enemies of *H. halys* and other pests have not been studied in Minnesota apple orchards. The purpose of this study was to characterize the composition of natural enemy communities, compare their abundances between apple cultivars, and assess their impact to *H. halys* sentinel eggs in Minnesota apple orchards. Results revealed differences in the relative abundance of taxa between sampling methods. In vacuum samples, arachnids, neuropterans, and coccinellids had the highest relative abundances. In yellow sticky traps, anthocorids were the most abundant. The total predator abundance did differ between the cultivars sampled across years. Results from sentinel egg mass surveys revealed low levels of predation and no parasitism across both years. Therefore, under the current standard management practices in conventional Minnesota apple orchards, the potential for biological control of *H. halys* appears low. Differences in predator abundance between cultivars, which may be due to the different growth vigor and growth habits of the cultivars, could be used to inform management decisions.

Introduction

Invasive insect pests in the United States (U.S.) account for approximately $13 billion in crop losses annually with an additional $500 million in pesticide treatments estimated for their control (Pimentel et al. 1997; Pimentel et al. 2005). The great success of these exotic species is generally accredited to the enemy release hypothesis, which posits that a species can become invasive by experiencing less regulation, than native species, by enemies in their introduced habitat and thus increase in abundance and distribution (Mack et al. 2000; Keane and Crawley 2002). However, natural enemies (i.e., arthropod predators and parasitoids) in the invaded ranges of invasive insects are being recognized as important agents for their biological control (Chang and Kareiva 1999; Symondson et al. 2002; Stilling and Cornelissen 2005).

The brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), is an invasive insect pest from east Asia that has made three separate introductions to the U.S. along the east and west coasts through means of global trade since the late 1990's (Hoebeke and Carter 2003; Leskey et al. 2012b; Valentin et al. 2017). The insect has since spread inward across the country and currently has been detected in 44 of 50 states (www.stopbmsb.org/whereis-bmsb/state-by-state/). *Halyomorpha halys* is polyphagous, feeding on many major fruit and vegetable crops in the U.S. (Kuhar et al. 2012; Leskey and Nielsen 2018), with apple being particularly susceptible (Leskey et al. 2012c). Injury by *H. halys* to apple results in feeding punctures and depressions on the exterior of the fruit and necrotic tissue immediately beneath the skin (Nielsen and Hamilton

2009; Acebes-Doria et al. 2016; Shanovich 2019), rendering it unmarketable for fresh market sale (Nielsen and Hamilton 2009; Leskey et al. 2012c). Insecticide use has increased substantially in response to *H. halys* presence in apple orchards due to the crop's low threshold for damage for fresh market sale. These additional sprays not only increase production costs, but also interfere with pre-existing integrated pest management programs and affect natural enemy populations (Leskey et al. 2012a). The negative impacts of broad-spectrum pesticides on natural enemies are well documented in many crop systems (Burn 1989; Theiling and Croft 1988, 1989; Epstein et al. 2000; Galvan et al. 2005; Deng et al. 2006; Mansfield et al. 2006; Rezaei et al. 2007; Sarvary et al. 2007; Johnson et al. 2008; Naranjo and Ellsworth 2009; Lu et al. 2012; Pezzini and Koch 2015).

In Minnesota, *H. halys* was first detected in 2010 (Koch 2014), with the presence of nymphs indicating established breeding populations (Koch 2014; Pezzini et al. 2019; MDA 2019). State-wide trapping and citizen reporting in Minnesota have shown continued increase of *H. halys* populations and occurrence of this pest in apple orchards (MDA 2019). *Halyomorpha halys* is thought to complete one full generation and a possible partial second generation per year in Minnesota. F_1 eggs appear from late April to early May and F_2 eggs appear from late July to early August. Each set hatches in one to two weeks depending on the ambient temperature experienced (Govindan, unpublished). *Halyomorpha halys* eggs are likely more vulnerable to attack by natural enemies than other, more mobile life stages of *H. halys* (Lee et al. 2014, Lee and Leskey 2015). Recently, there have been numerous efforts across the U.S. to characterize the composition

of arthropod natural enemy communities attacking *H. halys*, to determine their impact and evaluate the need for classic biological control (Cornelius et al. 2016a, b; Ogburn et al. 2016; Morrison et al 2016; Abram et al. 2017; Pezzini et al. 2018).

Extensive sampling in apple orchards has shown that the predatory arthropod community in apple tree canopies is highly variable (Oatman et al. 1964, McCaffrey and Horsburgh 1980, Carroll and Hoyt 1984, Miliczky and Horton 2005, Biddinger et al. 2012, Horton et al. 2012, Morrison et al. 2016), with their impacts to *H. halys* eggs varying by state within the U.S. (Ogburn et al. 2016). In addition to field surveys, laboratory trials have confirmed predation of *H. halys* by some commonly found generalist predators. Morrison et al. (2016) found that Tettigoniidae (Orthoptera) and Carabidae (Coleoptera) were the most frequent and efficient predators of *H. halys* eggs in laboratory trials, followed by Forficulidae (Dermaptera), Salticidae (Araneae), and Gryllidae (Orthoptera). Pote and Nielsen (2017) discovered that various abundant natural enemies demonstrate stage-specific predation of *H. halys*. Specifically, they found that Acrididae (Orthoptera) and Coccinellidae species (Coleoptera) reduced the hatch rate of *H. halys* eggs; hemipteran predators, such as *Nabis* spp. (Nadbidae: Hemiptera) and Reduviidae (Hemiptera) reduced survival of first instar *H. halys* nymphs; and *Nabis* spp. and *Podisus maculiventris* (Say) (Coccinellidae: Coleoptera) nymphs reduced survival of second instar *H. halys* nymphs.

Sentinel egg mass surveys have been widely used to quantify the potential impact of predation and parasitism on *H. halys* in North America. Total egg

mortality attributable to predation typically ranges from 5 to 25%, but may reach up to 83% in some cases (Abram et al. 2017). Levels of mortality from predators in agroecosystems have been found to vary by crop with apple having the highest level of attacked eggs in several studies (Morrison et al. 2016; Ogburn et al. 2016). In Minnesota, overall egg predation rates across soybean and forest habitats have been found to be 3.7% on average (Pezzini et al. 2018).

In the U.S., several species of indigenous egg parasitoids from three families have been found to parasitize wild and sentinel (both fresh and frozen) egg masses of *H. halys* in the field; with platygastrids in the subfamily Telenominae being the most prominent (Jones et al. 2014; Rice et al. 2014; Cornelius et al. 2016a, b; Herlihy et al. 2016; Pezzini et al. 2018). In the native range of *H. halys*, egg parasitoids from the genera *Trissolcus* (Hymenoptera: Platygastridae) and *Anastatus* (Hymenoptera: Eupelmidae) attack and suppress populations with parasitism rates estimated at 63 to 85% (Rice et al. 2014). However, adventive populations of *Trissolcus japonicus* (Ashmead) (Hymenoptera: Platygastridae), an egg parasitoid species of *H. halys* from its native range, have been detected in both the eastern and western US over consecutive years (Talamas et al. 2015; Milnes et al. 2016; Morrison et al. 2018). Modeling has also revealed that temperate regions of the U.S., such as the Midwest, have high climatic suitability to support the potential range expansion of *T. japonicus* (Avila and Charles 2018).

The abundance of pest species and their natural enemies are influenced not only by host plant species, but also cultivars within a species (Bergman and

Tingey 1979). Differential abundance of natural enemies among crop cultivars has been documented for many arthropod predators and parasitoids and is thought to be caused by several factors including plant morphology and variation in broadly toxic resistance factors, as many natural enemies use the plants as habitat and sources of water (Bergman and Tingey 1979).

We are unaware of studies that have characterized the natural enemy community in Minnesota apple orchards and assessed their impact to *H. halys* eggs. Furthermore, we are unaware of studies that have compared natural enemy abundances between apple cultivars. In this study, we examined the composition of predator taxa in Minnesota apple orchards and quantified their season-long abundances between two apple cultivars. To accomplish this, we documented natural enemies collected using vacuum sampling and yellow sticky traps from multiple conventional apple orchards in two popular Minnesota apple cultivars. We also examined the impact of natural enemies to *H. halys* eggs in Minnesota apple orchards via sentinel egg mass surveys. Results of this research will add valuable information to the growing list of natural enemy taxa catalogued in U.S. apple orchards, as community compositions are known to vary by state and crop (Ogburn et al. 2016). Knowledge of natural enemy taxa present and their collective impact to *H. halys* eggs will inform management decisions and the need for classical biological control in Minnesota. Finally, differences in predator abundance between cultivars could also be used to inform management decisions.

Materials and Methods

Field sites and apple cultivars

Sampling for natural enemies was conducted at apple orchards in southeastern Minnesota). In 2017, sampling was conducted at four conventional orchards; two located in Washington County and two in Dakota County, Minnesota; which will be referred to as WA-1, WA-2, DK-1 and DK-2, respectively. The cultivars Honeycrisp and Zestar!®, which represent two popular cold-hardy cultivars grown throughout Minnesota (Finnigan et al. 2000), were selected to be sampled at each orchard. In 2018, the DK-1 orchard location was dropped from all studies due to removal of their Zestar!® trees. All trees sampled were a minimum of 10 years old, on dwarfing rootstock (Table 2.1) and conventionally managed following standard recommendations and spray regimes for the area (Krawczyk and Biddinger 2018; Bordelon et al. 2019; Wise 2019).

Insect sampling

Within the orchards, arthropod predators were sampled via vacuum sampling the tree foliage and yellow sticky traps placed in the canopies, which have been both found to be effective methods for sampling natural enemies in trees (Legner and Oatman 1964; Basset 1988,; Basset et al. 1997; Mason 1992; Colunga-Garcia and Gage 1998; Gurr et al. 1999; Ozanne 2005; Sarvary et al. 2007; Wall and Shaw 2008; Yi et al. 2012).

For vacuum sampling, an 11.3-m^3/min (400-cfm) gas handheld blower vacuum (Homelite Consumer Products Inc., Anderson, SC) was used with the lower and upper tube nozzle attached. The vacuum was modified in a similar fashion to that described by Zou et al. (2016), by drilling a hole about three inches

down from the mouth of the upper nozzle attachment and inserting a large hexagonal screw into it. A five-gallon mesh paint strainer was inserted inside the nozzle and secured to the rim with an adjustable Velcro-elastic band fastened around the nozzle attachment under the screw to prevent the mesh from sliding up the nozzle and getting sucked into the vacuum during sampling (Figure 2.1). Within the apple orchards, vacuum sampling collections were made from 12 representative trees in each cultivar every other week July through mid-September in 2017 and from mid-June through mid-September in 2018. The foliage of each tree was vacuum sampled between one to three meters off the ground for two minutes total, one minute on each side, spending equal time on the inner and outer canopy leaves. After sampling each tree, the contents of the mesh strainer were emptied into a labeled resealable plastic bag.

For yellow sticky trap sampling, 15.2×30.5-cm Agrisense medium-yellow sticky strips (Great Lakes IPM, Vestaburg, MI) were cut in half to be 15.2×15.2 cm and hole punched (6.35-mm diameter holes) at both ends for use. Within the apple orchards, five randomly selected trees were chosen in each cultivar to hang a sticky card. Yellow sticky traps were secured in the tree canopies with zip ties about two meters off the ground towards the outer edge of the canopy to avoid leaves sticking to them (Figure 2.1). Yellow sticky traps were changed weekly. In 2017, yellow sticky trap sampling in orchards occurred from early August through mid-September and in 2018, from mid-June through mid-September. Upon collection, each yellow sticky trap was wrapped in clear plastic.

All sticky cards and vacuum samples were stored in a -18 °C freezer until they could be processed. All arthropod predator specimens were identified to the family level. The predator families with the highest relative abundance were identified for further analyses and will be referred to as “predominant groups”.

Sentinel egg mass survey

Frozen sentinel egg masses were prepared from *H. halys* egg masses that were collected from a laboratory colony originating from wild-collected individuals in Wyoming, Minnesota, in both November 2016 and 2017. Methods for rearing of *H. halys* and collection of egg masses followed those of Pezzini et al. (2018) and methods for preparation and deployment of egg masses were adapted from Tatman et al. (2013) and Pezzini et al. (2018). Prepared egg masses attached to cardstock were stored in a −80 ºC ultralow freezer for up to three months and removed two to four hours before placement in the field. Frozen *H. halys* sentinel eggs masses have been used in many studies to examine predation and parasitism by natural enemies (Herlihy et al. 2016; Morrison et al. 2016; Ogburn et al. 2016; Lara et al. 2016; Roversi et al. 2016; Pezzini et al. 2018). Furthermore, it has been shown that native parasitoids are more likely to develop and emerge from frozen rather than fresh *H. halys* sentinel egg masses, which could be due to lack of host defense mechanisms (Haye et al. 2015; Abram et al. 2016; Herlihy et al. 2016; Pezzini et al. 2018). Prior to deployment, the number of undamaged eggs in all egg masses was recorded.

Frozen sentinel egg masses were deployed every two weeks at the WA-1 and DK-1 orchards from late July to mid-September in 2017. In 2018, sentinel

egg masses were deployed every two weeks from mid-June to mid-September but were restricted to the WA-1 orchard (see Methods: Field Sites and Apple Cultivars). On each date of deployment, a single egg mass was deployed in each of 10 randomly selected trees in each cultivar in each orchard (20 egg masses per orchard). Egg masses were fastened to the underside of leaves using one plastic clothespin and one paperclip. After 72 hours, the sentinel egg masses were retrieved from the field, and the number of eggs and fates of retrieved eggs were recorded. Fates of retrieved eggs were categorized according to their predator damage syndrome as either exhibiting chewing damage or sucking damage (Morrison et al. 2016; Ogburn et al. 2016; Pezzini et al. 2018) by examining the eggs under a dissecting microscope. Eggs were classified as 'missing' when the cardstock was retrieved from the field with some or all eggs missing. Missing eggs were categorized as complete chewing, which may more accurately estimate predation on *H. halys* by chewing predators (Morrison et al. 2016; Ogburn et al. 2016; Pezzini et al. 2018).

Following examination, retrieved egg masses were stored separately in 2.5×7.0-cm plastic vials at ~25 ºC, 70% RH, and a 16:8 (L:D) h photoperiod for six weeks and monitored weekly for parasitoid emergence (Pezzini et al. 2018). Unhatched eggs were dissected to search for evidence of undeveloped parasitoid that failed to emerge (Tatman et al. 2013).

Statistical Analyses

All analyses were conducted with R version 3.4.4 (R Core Team 2018). We examined the relative abundances of collected families to determine the

predominant groups in Minnesota apple orchards. Methods for analysis of relative abundance were adapted from Pezzini et al. (2019) and performed separately for sampling methods and years. Friedman's nonparametric test (package, *code:* rcompanion, *friedman.test;* Mangiafico 2018) and Conover post-hoc test (package, *code*: PMCMR, *posthoc.friedman.conovertest*; Pohlert 2016) were used to compare relative abundance of all natural enemy families for each sampling method and year across orchards.

Because of the difference in sampling duration between apple cultivars (i.e., Zestar!® is harvested two weeks earlier than Honeycrisp in Minneosta), comparison of natural enemy abundance between cultivars and orchard locations was restricted to the initial sampling date through the harvest of the earlier maturing cultivar for each year. During exploratory data-analysis we did not detect strong temporal trends (e.g., linear, quadratic, exponential) for total natural enemy abundance or predominant groups, perhaps because biweekly samples were sufficiently independent. Linear mixed effect models with cultivar as main effect and tree nested in orchard as random effects were used to compare abundances of total natural enemies and predominant predator taxa per tree per sample date for vacuum samples and per tree per day for yellow sticky traps (package, code: lme4, *lmer*; Bates et al. 2015) and analyzed separately with one-way ANOVAs (package, code: lmerTEST, *anova*; Kuznetsova et al. 2019). Inclusion of a term for tree as a random effect accounted for repeated biweekly sampling of the trees. Square root transformations of the response variables were used to normalize error and satisfy the assumption of equal variance. If the

cultivar*location term was not significant ($P > 0.05$), it was removed from the model. Where differences existed according to the overall ANOVA for factors of interest ($\alpha = 0.05$), least-square means (package, code: lsmeans, *lsmeans*; Lenth 2016) were compared using Tukey's HSD test.

Comparison of egg predation between cultivars was also restricted to the initial sampling date through the harvest of the earlier maturing cultivar for 2017; data from 2018 was excluded from analysis as the study was unreplicated due to only one orchard being sampled (see Methods: Field Sites and Apple Cultivars). Egg predation was examined as the proportion of deployed eggs affected by predation (i.e., chewing and sucking predation combined) (Cornelius et al. 2016b, Morrison et al. 2016, Ogburn et al. 2016, Pezzini et al. 2018). Generalized linear models with a binomial response were used to compare proportions of egg outcomes (i.e., eggs predated vs. not predated) with cultivar as the main effect and location as a random effect (package, code: lme4, *glmer*; Bates et al. 2015) and analyzed with one-way ANOVAs (package, code: car, *Anova*; Fox et al. 2019). Analyses were not performed for each type of predation (e.g., chewing, sucking), because the rates of each were relatively low.

Results

Orchard sampling

Across cultivars, sampling methods and years, a total of 19 families of predators were collected (Table 2.1) for a total of 3,653 specimens from 1,242 sample units. There were significant differences among the relative abundances of predator families for each year and sampling method (Table 2.1). In vacuum

samples from 2017, arachnids in the families Araneidae and Salticidae were the most abundant, comprising 70% of all specimens collected, followed by insects in the families Coccinellidae (11.6%) and Hemerobiidae (10.4%). In yellow sticky traps from 2017, insects in the family Anthocoridae were the most abundant comprising over 70% of all specimens, followed by Syrphidae (32%) and Hemerobiidae (6%), with overall low numbers of arachnid specimens. In vacuum samples from 2018, arachnids in the families Linyphiidae, Araneidae, and Salticidae were the most abundant, together comprising over 50% of all specimens; insects in the families Anthocoridae (7.6%), Coccinellidae (5.4%), and Hemerobiidae (5.3%) followed in relative abundance. In yellow sticky traps from 2018, insects in the family Anthocoridae were again the most abundant, comprising over 80% of all specimens collected, followed by arachnids in the family Thomisidae (3%) and insects in Coccinellidae (2.9%). Based on these results, we decided to group some of the most abundant related families into predominant groups for use in further analyses. For vacuum samples, predominant groups were Arachnida, Neuroptera and Coccienllidae and for yellow sticky traps the predominant group was Anthocoridae. We refrained from analyzing Syrphidae and Arachnida as predominant groups for yellow sticky traps as their relative abundances were not consistently high across years (Table 2.2).

For vacuum samples from 2017, total predator abundance was greater on Zestar!® than Honeycrisp (Table 2.3, Figure 2.1a). For vacuum samples from 2018, total predator abundance did not differ between cultivars (Table 2.3, Figure 2.1a). Similar trends were observed for the predominant taxa. The abundance of

arachnids was greater on Zestar!® than Honeycrisp in 2017, but did not differ in 2018 (Table 2.3, Figure 2.1b). The abundance of coccinellids was greater on Zestar!® than Honeycrisp in both 2017 and in 2018 (Table 2.3, Figure 2.1c). The abundance of neuropterans was greater on Zestar!® than Honeycrisp in 2017, but not in 2018 (Table 2.3, Figure 2.1d). For yellow sticky traps, total predator abundance was greater on Zestar!® than Honeycrisp in both 2017 and 2018 (Table 2.3, Figure 2.2a). The abundance of anthocorids was greater on Zestar!® than Honeycrisp in 2017, but did not differ in 2018 (Table 2.3, Figure 2.2b).

Sentinel egg mass survey

Across both years, a total of 200 *H. halys* frozen sentinel egg masses were deployed at the two apple orchard locations, consisting of a total of 5,571 eggs (Table 2.4) with an average of 27.9 (±0.2 SEM) eggs per egg mass. All the egg masses were recovered each year. Across all eggs, no parasitoids emerged and there was no evidence of incomplete parasitoid development. Of the total number of eggs deployed in 2017, only 28 eggs, or 1.3% were affected by predation (Table 2.4). Overall, in 2017, 18 eggs (0.8%) were affected by chewing predation and 10 eggs (0.5%) were affected by sucking predation (Table 2.4). Of the total number of eggs deployed in 2018, 84 eggs, or 2.6% were affected by predation (Table 2.4). Overall, in 2018, 51 eggs (1.5%) were affected by chewing predation and 33 eggs (1.0%) were affected by sucking predation (Table 2.4). In 2017, from 28 July through 28 August, the proportion of eggs predated did not differ between cultivars ($LR\ \chi^2 = 0.412$, df=1, P=0.521), with 1.4% of eggs being predated in Honeycrisp and 1.1% of eggs being predated in Zestar!®.

Discussion

Natural enemy community compositions in apple orchards have been found to differ by state (Oatman et al. 1964, McCaffrey and Horsburgh 1980, Carroll and Hoyt 1984, Miliczky and Horton 2005, Biddinger et al. 2012, Horton et al. 2012, Morrison et al. 2016) and their impact to *H. halys* eggs are known to vary by crop and state as well (Morrsion et al. 2016; Ogburn et al. 2016). Additionally, preference among crop cultivars has been documented for many natural enemies (Bergman and Tingey 1979). To our knowledge, our study provides the first characterization of the composition of arthropod predator taxa in Minnesota apple orchards and quantification of their impact to *H. halys* eggs. Additionally, to our knowledge, we provide the first documentation of arthropod predator abundances varying between apple cultivars. Conducting these studies in commercial apple orchards provided an opportunity to measure the potential impact of biological control under typical production conditions for the state.

Across years and sampling methods, we found predator abundance to be higher in Zestar!® than Honeycrisp apple trees, with the exception of vacuum samples in 2018. This phenomenon could be due to a number of factors. First, differential orientation of predators to the host plants of their prey, which can be caused by characteristics of the host plant, including morphology and secondary metabolites, could be causing this difference among cultivars (Bergman and Tingey 1979). For example, Franklin and Holdaway (1966) reported differential attraction of *Lydella grisescens* Robineau-Desvoidy (Diptera: Tachinidae), a parasitoid of the European corn borer, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae), to different corn hybrids with the same host densities. Honeycrisp

trees are known to exhibit a somewhat spreading growth habit with low to moderately vigorous growth annually, while Zestar!® trees exhibit an upright, spreading growth habit with moderate to highly vigorous growth annually (https://mnhardy.umn.edu/varieties/fruit/apples). All trees included in our study were grown on dwarfing rootstocks and were a minimum of 10 years old (Table 2.1). Therefore, we suspect the more vigorous annual growth and/or growth habit of Zestar!® trees may be favorable to arthropod predators and their prey compared to Honeycrisp trees. Secondly, this difference could be attributed to potential differences in prey abundances between the two cultivars, but this was not quantified in our study.

There are few studies on natural enemies in apple orchards available from close proximity to Minnesota to compare our community composition results. Cleveland and Hamilton (1958) in southern Indiana, found Chrysopidae to be the most abundant family of predators, followed by Miridae and Syrphidae, respectively. Oatmen et al. (1964) in northeast Wisconsin, found predators in the families Miridae, Coccinellidae and Chrysopidae to be the most abundant. While Chrysopidae was not one of the most abundant families in our study for either year or sampling method, another neuropteran family, Hemerobiidae, was in the top three most abundant insect families in our study for vacuum samples in both 2017 and 2018 and for yellow sticky traps in 2017 (Table 2.2, Figure 2.1), but only found in very low numbers in Indiana (Cleveland and Hamilton 1958). Coccinellidae was the second most abundant insect family for vacuum samples in both 2017 and 2018 and for yellow sticky traps in 2018. Interestingly, we found

no Miridae in our samples from either year (Table 2.2). We found high numbers of salticids in vacuum samples both years (Table 2.2). Likewise, other surveys of predatory arthropods in apple that have included arachnids have consistently found Salticidae to be one of the most abundant families collected (McCaffrey and Horsburgh 1980; Miliczky and Horton 2005; Horton et al. 2012; Morrison et al. 2016). However, none of these similar studies have found arachnids in the family Araneidae to be highly abundant in apple orchards, while we found them to be relatively abundant in both 2017 and 2018 vacuum samples (Table 2.2).

The relative composition of predator communities varied between sampling methods in our study. In each year, arachnids comprised the majority of specimens collected in vacuum samples and anthocorids comprised the majority of specimens collected in yellow sticky traps. This difference in composition of taxa collected between the two sampling methods was expected as many studies have found different non-destructive arthropod sampling methods are biased towards certain taxonomic groups and recommend using multiple methods to estimate community assemblages (Basset et al. 1997; Green 1999; Ozanne 2005; Doxon et al. 2011; Bannerman et al. 2015). Vacuum sampling has previously been found to be effective for capturing arachnids in orchard tree canopies (Green 1999). Furthermore, yellow sticky traps have been previously found to be effective at capturing anthocorid species in other crops (Musser et al. 2004; Schmidt et al. 2008; Atakin and Bayram 2011; Akhtar et al. 2013).

Overall, we found the impact of natural enemies on sentinel eggs of *H. halys* to be low, amounting to an average of only 2.0% predation and no

parasitism of the deployed eggs across cultivars and years. This rate of *H. halys* egg mortality by natural enemies was lower than that observed in similar studies conducted throughout the eastern US, including Minnesota (Cornelius et al. 2016b; Ogburn et al. 2016; Morrison et al 2016; Abram et al. 2017; Pezzini et al. 2018). In a meta-analysis of *H. halys* sentinel egg mass studies in North America and Europe, Abram et al. (2017) found predation rates of ~20% on frozen *H. halys* eggs. In addition, they found that parasitism levels of *H. halys* egg were extremely variable, ranging from 0-59% when based solely on parasitoid emergence; when accounting for the additional impact of unemerged parasitoids from egg dissections, the average increase in parasitism was 6.16 ± 1.52% (Abram et al. 2017). In Minnesota, relatively low levels of mortality due to predation and parasitism of *H. halys* eggs were found in soybean (3.7%) and forest (0.4%) habitats (Pezzini et al. 2018). A two-year study comparing predation and parasitism in various organic crops throughout the eastern U.S. found the highest rates of egg mortality in apple (~30% across years) compared to other crops (Ogburn et al. 2016). The rates of egg mortality in those organic apple orchards (Ogburn et al. 2016) were higher than those reported here, which may be partially due to the use of insecticides affecting natural enemy populations in the conventional orchards we sampled.

We did not find an effect of cultivar on rates of sentinel egg mass predation in 2017. This was somewhat unexpected as natural enemy abundance was generally found to be greater in Zestar!® than in Honeycrisp (Figure 2.1, 2.2). However only two orchard locations (WA-1 and DK-1) were surveyed with

sentinel egg masses. This result could be explained by more efficient predators of *H. halys* eggs not being present in Minnesota apple orchards. Morrison et al. (2016) found the most frequent and efficient predators of *H. halys* eggs to be Tettigoniidae and Carabidae followed by Forficulidae, Salticidae and Gryllidae in laboratory trials and confirmed their presence in West Virginia apple orchards. We did not find any Tettigoniidae species in any of our samples, and low numbers of Carabidae, Forficulidae and Gryllidae.

With populations on the rise, *Halyomorpha halys* poses a threat to Minnesota apple production (MDA 2019). Understanding the natural enemy community composition and its impact on *H. halys* in Minnesota apple orchards provides insight into the potential for biological control of this pest. Additionally, understanding factors, such as cultivar, that influence natural enemy abundance could influence management strategies. The findings of our study indicate that with current standard management practices in conventional Minnesota apple orchards, control of *H. halys* is likely to be low by existing natural enemies. Additional control tactics, such as conservation biological control and the introduction of *T. japonicus*, should be considered in Minnesota as populations of *H. halys* continue to rise. Differences in predator abundance between cultivars, which may be due to the different growth vigor and habits of the cultivars, could be used to inform management decisions. More scouting could be focused on cultivars, like Honeycrisp, with lower predator abundance or could be used to inform future planting decisions. These findings also inform future work on the potential for biological control of pentatomid pests, as their communities change

and increase in abundance in the region (Hunt 2001, 2014; Michel et al. 2013; Swanson and Keller 2013; Koch 2014; Koch et al. 2017; Koch et al. 2018; Pezzini et al. 2019).

Table 1 Tree age and rootstocks for apple cultivars sampled at the four different orchard locations.

Cultivar		Orchard[a]			
		DK-1	DK-2	WA-1	WA-2
Honeycrisp					
	Tree age[b]	14	12	15	18
	Rootstock[c]	M7	M26	B9	M7
Zestar!®					
	Tree age	16	12	10	18
	Rootstock	B9	M26	B9	M7

[a]Orchards abbreviated as DK-1 and DK-2 for two orchards located in Dakota County, Minnesota and WA-1 and WA-2 for two orchards located in Washington County, Minnesota

[b] Tree age measured in years since initial planting as of 2017

[c] Roostock abbreviated as M for Malling and B for Budagagovsk

Figure 2.1. Images of sampling methods used for predator sampling and measuring their impact to *H. halys* eggs in apple tree canopies: a) vacuum sampling with a modified garden blow-vac, b) yellow sticky traps secured with zip ties about two meters off the ground towards the outer edge of the canopy, and c) sentinel egg mass of *H. halys* affixed to cardstock and fastened to the underside of leaves using one plastic clothespin and one paperclip

Table 2 Season-long total number of individuals and relative abundance (RA) for each predator taxon encountered in four Minnesota apple orchards in 2017 and three orchards in 2018 for two sampling methods.

	Vacuum samples				Yellow sticky traps			
	2017		2018		2017		2018	
Taxa	Total	RA(%)	Total	RA(%)	Total	RA(%)	Total	RA(%)
Araneae								
Araneidae	336	0.507**a**	582	0.200**ad**	9	0.042**bc**	4	0.001**b**
Clubionidae	--	--	167	0.057**bf**	--	--	1	0.000**b**
Linyphiidae	--	--	586	0.202**d**	--	--	31	0.010**abc**
Oxyopidae	--	--	76	0.026**efg**	--	--	4	0.001**b**
Philodromidae	--	--	162	0.056**bc**	--	--	--	---
Salticidae	128	0.193**ab**	365	0.126**acd**	7	0.032**bcd**	63	0.021**ac**
Theridiidae	--	--	23	0.008**eg**	--	--	16	0.005**bc**
Thomisidae	--	--	223	0.077**abc**	--	--	88	0.030**ac**
Coleoptera								
Carabidae	21	0.032**bcde**	8	0.003**e**	7	0.032**bd**	7	0.002**bc**
Coccinellidae	77	0.116**ab**	158	0.054**bc**	7	0.032**bc**	87	0.029**ac**
Dermaptera								
Forficulidae	1	0.002**e**	13	0.004**e**	1	0.005**e**	--	--
Diptera								
Syrphidae	22	0.033**abcde**	17	0.006**eg**	69	0.319**a**	19	0.006**bc**
Hemiptera								
Anthocoridae	35	0.053**abc**	221	0.076**abc**	161	0.745**a**	2512	0.848**a**
Nabidae	--	--	12	0.004**e**	--	--	1	0.000**b**
Reduviidae	2	0.003**e**	5	0.002**e**	--	---	4	0.001**b**
Neuroptera								
Chrysopidae	37	0.056**abcd**	82	0.028**bfg**	--	--	30	0.010**bc**
Hemerobiidae	69	0.104**ab**	153	0.053**abc**	13	0.060**c**	62	0.02**ac**
Opiliones								
Sclerosomatidae	6	0.009**de**	29	0.010**e**	3	0.014**de**	29	0.010**bc**
Orthoptera								
Gryllidae	6	0.009**cde**	24	0.008**e**	2	0.009**e**	4	0.001**b**
Chi-square		31.65		70.91		34.23		58.87
df		11		18		9		16
P		0.001		<0.001		<0.001		<0.001

* Letters indicate results from Conover post-hoc test following Friedman's tests for differences between taxon relative abundances

Table 2.3. Analysis of variance for the effect of cultivar on the mean number of predators and predominant groups per tree per sampling date for vacuum samples and for yellow sticky traps in 2017 and 2018.

Sampling method	Predators	Year	*F*-value	DF	*P*-value
Vacuum	Total	2017	20.774	1, 252	<0.001
		2018	0.567	1, 435	0.435
	Arachnida	2017	8.643	1, 252	0.004
		2018	0.236	1, 435	0.628
	Neuroptera	2017	4.816	1, 252	0.029
		2018	0.065	1, 435	0.799
	Coccinellidae	2017	17.79	1, 249	<0.001
		2018	3.893	1, 580	0.048
Sticky traps	Total	2017	34.169	2, 49	<0.001
		2018	5.083	4, 286	0.025
	Anthocoridae	2017	11.089	2, 49	0.002
		2018	2.373	4, 286	0.125

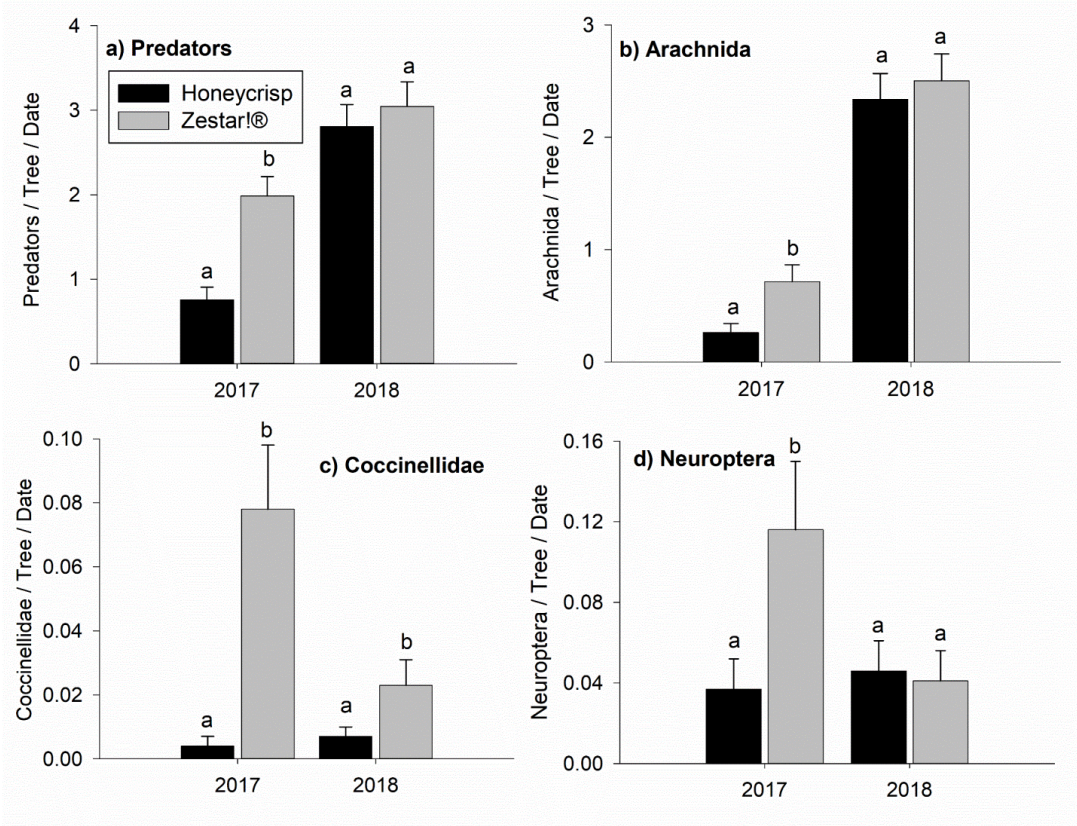

Figure 2.2 Mean (+CI) number of predators and the three most abundant predator taxa per tree per sampling date from vacuum samples in Minnesota apple orchards in 2017 and 2018. Different letters indicate a significant difference between cultivars within years at

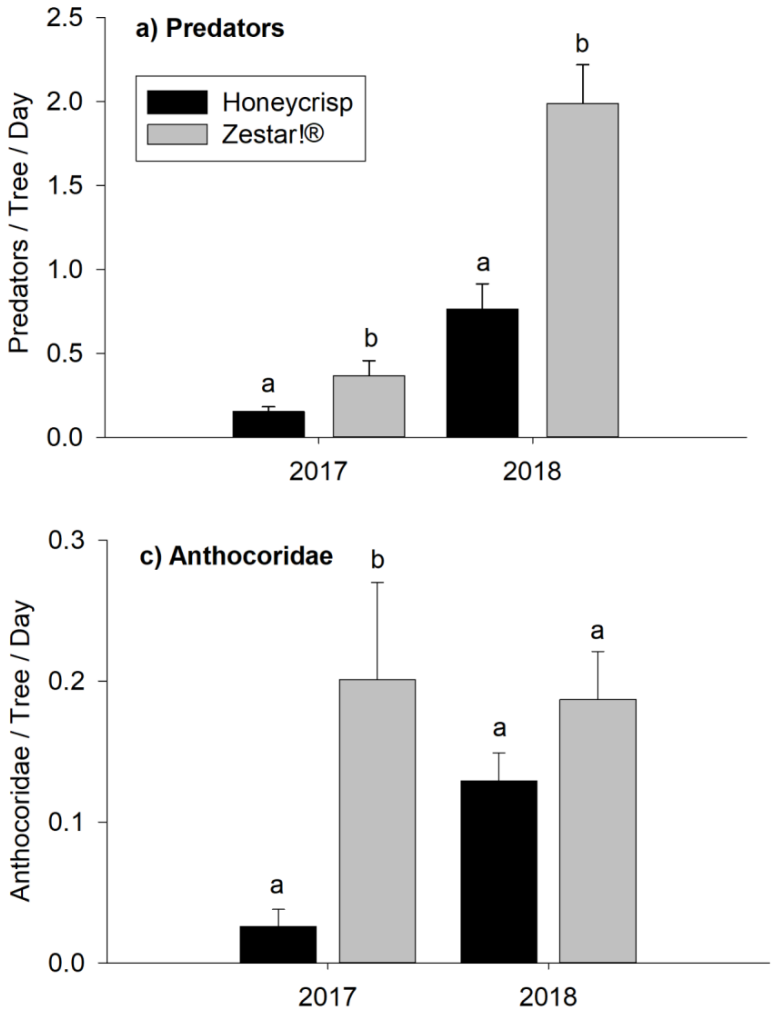

Figure 2.3 Mean (+CI) number of predators and the most abundant groups per trap per day from sticky card samples in Minnesota apple orchards in 2017 and 2018. Different letters indicate a significant difference between cultivars within years at $P < 0.05$.

Table 2.4. Recovery of *H. halys* sentinel eggs deployed in two apple cultivars (Honeycrisp and Zestar) in two orchards from 28 July 2017 – 31 August 2017 and one orchard from 15 June 2018 – 31 August 2018.

Location	Year	Fates of Recovered Eggs* (no. egg masses: no. eggs)		
		Chewing	Piercing-sucking	Intact
WA 1	2017			
Honeycrisp		2: 6	7: 9	20: 537
Zestar!®		2: 8	0: 0	20: 551
DA 1				
Honeycrisp		0: 0	1: 1	20: 559
Zestar!®		2: 4	0: 0	20: 561
WA 1	2018			
Honeycrisp		7: 35	12: 16	60: 1614
Zestar!®		10: 16	11: 17	60: 1638

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